Nucleic acids which are hybridizable to an nucleic acid comprising a nucleotide sequence encoding the chimeric protein disclosed in Section 4.2., or any fragments, analogues or derivatives thereof, can be isolated, by nucleic acid hybridization under conditions of low, high, or moderate stringency (*See also* Shilo and Weinberg, 1981, *Proc. Natl. Acad. Sci. USA*, 78:6789-6792).

4.2. CHIMERIC PROTEIN

The present invention provides a chimeric protein comprising, from N-terminus to C-terminus: a) a first peptidyl fragment consisting of an amino acid sequence that has at least 40% identity to a domain containing at least first 20 N-terminal amino acids of human growth hormone (hGH) protein, in which the percentage identity is determined over an amino acid sequence of identical size to the domain of hGH; b) an Arg residue, or a Lys residue, or a second peptidyl fragment consisting of at least 2 amino acids in which peptidyl fragment the most C-terminal amino acid residue is an Arg or a Lys; and c) a third peptidyl fragment consisting of an amino acid sequence containing more than two cysteine (Cys) residues which peptidyl fragment is not a portion of hGH protein.

In a preferred embodiment, the present invention provides a chimeric protein described above, wherein the first peptidyl fragment consists of an amino acid sequence that has at least 60% identity to the domain of hGH protein.

In another preferred embodiment, the present invention provides a chimeric protein described above, wherein the first peptidyl fragment is capable of being bound by an anti-hGH antibody.

In a more preferred embodiment, the present invention provides a chimeric protein described above, wherein the first peptidyl fragment consists of the amino acid sequence of SEQ ID NO:1.

In another more preferred embodiment, the present invention provides a chimeric protein described above, wherein the first peptidyl fragment consists of the amino acid sequence of SEQ ID NO:2.

In a preferred embodiment, the present invention provides a chimeric protein described above, wherein the second peptidyl fragment consists of the amino acid sequence of SEQ ID:3.

In a specific embodiment, the present invention provides a chimeric protein described above, wherein the third peptidyl fragment is an insulin precursor.

In a preferred embodiment, the present invention provides a chimeric protein described above, wherein the insulin precursor is of human origin.

In a more preferred embodiment, the present invention provides a chimeric protein

described above, wherein the human insulin precursor is capable of being bound by an anti-human-insulin antibody.

In another more preferred embodiment, the present invention provides a chimeric protein described above, wherein the human insulin precursor consists of the amino acid sequence of SEQ ID NO:4.

In still another more preferred embodiment, the present invention provides a chimeric protein described above, wherein in the human insulin precursor, B chain and A chain of the human insulin precursor are separated by an amino acid residue or a peptidyl fragment consisting of 2 to 34 amino acid residues.

In yet another more preferred embodiment, the present invention provides a chimeric protein described above, wherein the human insulin precursor consists of the amino acid sequence of SEQ ID NO:5.

In a most preferred embodiment, the present invention provides a chimeric protein consisting of the amino acid sequence of SEQ ID NO:6.

In another most preferred embodiment, the present invention provides a chimeric protein consisting of the amino acid sequence of SEQ ID NO:7.

4.3. OBTAINING CHIMERIC DISCLOSED IN SECTION 4.2.

Chimeric proteins disclosed in Section 4.2., and derivatives, analogues and fragments thereof can be obtained by any method known in the art, including but not limited to recombinant expression methods, purification from natural sources, and chemical synthesis.

For example, chimeric proteins disclosed in Section 4.2. can be obtained by recombinant protein expression techniques. For recombinant expression, the gene or portion thereof encoding chimeric proteins disclosed in Section 4.2. is inserted into an appropriate cloning vector for expression in a particular host cell. A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such as pBR322 or pUC plasmid derivatives or the Bluescript vector (Stratagene). The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. However, if the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may

comprise specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.

In an alternative method, the desired gene may be identified and isolated after insertion into a suitable cloning vector in a "shot gun" approach. Enrichment for the desired gene, for example, by size fractionation, can be done before insertion into the cloning vector.

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated gene encoding chimeric proteins disclosed in Section 4.2., cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene may be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

The nucleotide sequence coding for chimeric proteins disclosed in Section 4.2., and derivatives, analogues and fragments thereof, or a functionally active analogues or fragments or other derivatives thereof, can be inserted into an appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. A variety of host-vector systems may be utilized to express the protein-coding sequence. These include but are not limited to mammalian cell systems infected with virus (*e.g.*, vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (*e.g.*, baculovirus); microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage DNA, plasmid DNA. or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

Any of the methods previously described for the insertion of DNA fragments into a vector may be used to construct expression vectors containing a chimeric gene consisting of appropriate transcriptional/translational control signals and the protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleic acid sequence encoding chimeric proteins disclosed in Section 4.2., and derivatives, analogues and fragments thereof, may be regulated by a second nucleic acid sequence so that the chimeric proteins disclosed in Section 4.2. are expressed in a host transformed with the recombinant DNA molecule. For example, expression of the chimeric proteins disclosed in Section 4.2. may be controlled by any promoter/enhancer element known in the art. Promoters which may